RESEARCH PAPER

Synergistic Immunomodulatory Effects of Dhatura Metel (Datura) and Tephrosia Purpurea in Dncb-Induced Chronic Eczema

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Received: 16th Dec, 2025; Revised: 8th Feb 2026; Accepted: 12th Feb, 2026; Available Online: 30th March, 2026

ABSTRACT
The skin condition atopic dermatitis, also known as chronic eczema, presents a long-term inflammatory skin disease which results from immune system problems and T-cell abnormalities and cytokine disturbances and skin damage and oxidative stress. The current treatment methods using corticosteroids and immunosuppressive agents help patients with symptoms, but these medications cause negative side effects when patients need to use them for extended periods so researchers seek alternative treatments which are both safe and affect multiple disease targets. The research examined how Datura metel leaf ethanolic extract and Tephrosia purpurea root ethanolic extract affect immune system function in rats which developed chronic eczema following DNCB exposure. The study used male albino rats which received five different treatment groups (n = 6/group) that included normal control and three experimental groups: DNCB-induced untreated and single-extract treated (Datura metel or Tephrosia purpurea) and combined treatment. The researchers administered oral extracts to subjects for 28 days through two different dose levels which used 10 mg/kg/day Dhatura metel and 200 mg/kg/day Tephrosia purpurea at a 1:20 ratio. The study performed Immunological assessment through three tests which measured T-cell proliferation after Concanavalin-A stimulation and T-cell ratio between $CD4^+$ and $CD8^+$ cells and measured IL-4 and IL-13 and IL-17 cytokines in blood serum and evaluated oxidative stress and performed histopathological examination. The DNCB induction process resulted in increased T-cell proliferation and higher Th2 cytokine production but it decreased IL-17 levels. The combined extract treatment approach successfully decreased T-cell proliferation while it brought back normal $CD4^+/CD8^+$ ratios and it decreased IL-4 and IL-13 levels while it restored IL-17 production and it decreased lipid peroxidation while it boosted antioxidant defenses and it fixed epidermal structure without causing any adverse effects. The research shows that Dhatura metel and Tephrosia purpurea therapy work together to create enhanced immunomodulatory effects which help T-cells achieve balance and maintain proper cytokine levels and skin health. The research establishes the first experimental proof which supports using multiple plant-based treatments to control chronic eczema over an extended period.
Keywords: *Eczema, DNCB, Immunomodulators, T-cell activity, Cytokine, Dhatura metel, Tephrosia purpurea, Alkaloids.*
How to cite this article: Kumari S, Singh VP, Synergistic Immunomodulatory Effects of Dhatura Metel (Datura) and Tephrosia Purpurea in Dncb-Induced Chronic Eczema. Int J Drug Deliv Technol. 2026;16(3): 234-240. DOI: 10.25258/ijddt.16.3.29
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

The skin condition atopic dermatitis (AD) also known as chronic eczema presents as a persistent inflammatory disorder which causes skin barrier damage and continuous itching and inflammation [1-2]. Eczema affects millions of people worldwide and has a major negative impact on quality of life because to its physical and psychological effects. The disease exists with multiple factors which include inherited conditions and immune system breakdowns and outside influences that affect its development [3–4]. The immune system dysfunction includes T-helper cell overactivation which leads to Th2 and Th17 subset expansion that produces IL-13 and IL-17 and IL-4 cytokines which sustain the inflammatory process and skin tissue destruction [5–6]. The treatment of autoimmune diseases through immunosuppressants and biologics and corticosteroids offers pain relief but these medications create multiple adverse effects and they are expensive and their therapeutic value decreases over time [7]. The current treatment challenges for eczema patients require researchers to develop new therapeutic methods which target the core immune system problems that cause the condition [8]. The therapeutic potential of bioactive chemicals exists in natural products which derive from medicinal plants. Dhatura metel and Tephrosia purpurea are two of the many plants with therapeutic qualities that

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have drawn notice for their long-standing applications in the treatment of inflammatory and immune-mediated diseases [9–10]. The plants contain phytochemicals which possess anti-inflammatory and antioxidant and immunomodulatory properties that help treat the immunological issues which occur in eczema patients. Dhatura metel: The Solanaceae family includes the thorn apple, a plant commonly used in traditional African and Asian medical systems. The plant contains three main groups of compounds which include flavonoids and phenolic compounds and strong alkaloids that include atropine and scopolamine [11]. The preclinical research of bioactive compounds showed their potential to function as strong immunosuppressants and anti-inflammatory agents. Studies indicate that Dhatura metel extracts affect immune system operation through decreased inflammatory cytokine production which enables their use for treating T-cell-related eczema symptoms [12–13]. The Fabaceae family includes Tephrosia purpurea which is a leguminous plant that people have used throughout history to treat different medical conditions such as skin diseases and liver disorders and breathing issues [14]. The bioactive compounds in this plant consist mainly of flavonoids and rotenoids and glycosides which demonstrate strong antioxidant and anti-inflammatory properties. Research indicates Tephrosia purpurea works as an effective treatment for chronic inflammatory diseases because it strengthens Treg cell function and stops Th17 inflammatory reactions which results in immune system balance [15]. Essential to the pathogenesis of eczema are T-cell subsets. Th2 cells, which produce IL-4 and IL-13, which activate mast cells and produce IgE, are the main cause of inflammation during the acute phase of the disease [16]. The body produces Th1 and Th17 cells which release IL-17 and interferon-gamma to cause tissue damage and inflammation during the chronic phase. People with eczema have an immune system that contains defective regulatory T-cells which function as immune regulators to control immune responses and contribute to the development of their condition. The immunological pathways which cause T-cell homeostasis disruption can be modified through specific treatments to provide patients with enduring relief from their eczema symptoms [17]. The immune system problems which occur in eczema patients can be treated through natural immunomodulator therapies which use Datura metel and Tephrosia purpurea as their components. Plant-based therapies influence a variety of immunological and inflammatory pathways, in contrast to synthetic medications that usually target a single pathway [18]. The flavonoids in Tephrosia purpurea and the alkaloids in Datura metel function as complete immune system regulators which reduce oxidative stress while they boost Treg cells and reduce inflammatory cytokine production. Scientists who study immunology and phytochemistry have identified the specific molecular mechanisms which plants use to produce their therapeutic compounds. Research studies have proven that Datura metel and Tephrosia purpurea demonstrate preclinical potential to treat essential inflammatory factors which cause eczema to develop [19]. Examples of cytokines that have been demonstrated to be inhibited by Datura metel extracts include TNF-alpha (TNF-03b1) and IL-6, whereas Tephrosia purpurea has demonstrated the capacity to decrease Th17-driven inflammation and support Treg-mediated immune regulation [20]. Research shows that Dhatura metel and Tephrosia purpurea contain distinct substances which impact immune system operation and decrease inflammation but scientists have not studied their ability to manage chronic eczema by regulating T-cells and balancing cytokines. The research establishes a new finding which shows how Datura metel and Tephrosia purpurea work together to treat chronic eczema in DNCB-induced models through their combined effects on Th2/Th17 cytokines and T-cell growth and skin tissue recovery and oxidative stress reduction. The treatment method uses multiple plant-based compounds to create a new approach which targets the underlying mechanisms of chronic inflammatory skin diseases for extended control.

MATERIALS AND METHODS

1. Plant Material Collection and Authentication

Tephrosia purpurea and Dhatura metel, two medicinal herbs, were collected from Jaipur, Rajasthan. Following a careful investigation, specialists from the University of Rajasthan confirmed their identities. These plants were assigned distinct RUBL codes: 21680 for Dhatura and 21682 for Tephrosia.

The verified plant specimens have been preserved as certified samples for use as references and in upcoming scientific research.

2. Preparation of Plant Extracts

The plant materials, Tephrosia purpurea root and Dhatura metel leaves, were treated independently. The washing procedure for samples eliminated contaminants before shade drying which helped maintain their phytochemical compounds. Each plant material was dried and then ground into a fine powder with an electric grinder. The powdered samples required sealed hygienic containers for storage because this method protected them from moisture and contaminants which made them suitable for future extraction procedures.

3. Extraction Method:

Tephrosia purpurea roots and Dhatura metel leaves were extracted independently using a Soxhlet device and ethanol solvent. order to optimize the yield of bioactive chemicals, each plant material was continuously extracted using heat for 8 to 10 hours. To concentrate the extracts, the ethanol solvent was subsequently removed using a rotary evaporator set to a lower pressure. For later examination and experimental usage, the resultant crude extracts were kept in airtight containers at 4°C.

PHYTOCHEMICAL ANALYSIS

A range of bioactive substances were identified through qualitative phytochemical screening of Tephrosia purpurea and Datura metel extracts. The tropane alkaloids scopolamine and atropine and hyoscyamine present in the

sample indicate strong immunomodulatory effects which result from their ability to control cholinergic pathways that regulate immune system function. The immune system receives advantages from flavonoids which contain quercetin and kaempferol because these compounds have anti-inflammatory and antioxidant properties which help them manage immune cell activities. Tannins contain antioxidant and antibacterial properties which help manage cytokine activity while protecting against further bacterial infections. The research proved saponins function as immune-enhancing compounds which activate macrophages and regulate their cytokine production patterns. The research showed that tephrosin glycosides present in glycosides protect skin health while maintaining equilibrium in the immune system. The bioactive compounds in these extracts function as a team to reduce oxidative damage while they stop inflammation and speed up tissue healing which makes them effective for managing chronic inflammatory skin diseases. The research investigated qualitative phytochemical screening as its main objective while identifying six immunomodulating and antioxidant compounds which include alkaloids and flavonoids and phenolics and tannins and saponins and glycosides. Research studies show that these compounds control cytokine signaling and oxidative stress pathways through dose-dependent mechanisms. The research needs future studies which will use quantitative phytochemical profiling and chromatographic characterization to prove how particular phytoconstituents affect immunological results. The bioactive compounds found in Tephrosia purpurea and Datura metel show potential for treating eczema and other chronic inflammatory skin diseases because they affect multiple targets which help regulate the immune system and protect tissues. The therapeutic effects become evident through multiple immunologically active compound classes which were identified through qualitative methods although quantitative phytochemical estimation was not performed. The multiple compounds in plant-based immunomodulators work together to produce their effects which match the complete drug responses seen in models of chronic inflammatory diseases.

Experimental Animals

30 male albino rats weighing 150–200 grams at 12 weeks were acquired for the study from a breeder that holds CPCSEA certification. The laboratory maintained a controlled environment for mice which included a 12-hour light/dark cycle and temperatures between 22±2°C. They had free access to water and standard lab food during the study period. The Institutional Animal Ethics Committee (IAEC) granted permission (IAEC approval number: NIMSUR/IAEC-01/2024/03) for this research to assess animal welfare standards and the study followed all ethical guidelines throughout the investigation.

Chemicals and Reagents

2,4-Dinitrochlorobenzene (DNCB), Concanavalin-A (ConA), ethanol (analytical grade), and all other reagents used in the study were of analytical grade and procured from standard certified suppliers. The ELISA kits available for commercial purchase served to measure cytokines (IL-4, IL-13 and IL-17) through procedures which followed the instructions provided by the manufacturers. The researchers prepared all biochemical reagents for oxidative stress assessment immediately before starting their experiments.

Induction of Eczema in rat

The sensitization phase requires a 1% DNCB solution which needs to be injected subcutaneously into the dorsal skin area for creating eczema through 50–100 µL volumes. 0.5% DNCB is given topically every other day for two weeks to keep eczema-like symptoms. The dosage and time can be changed according to the needs of the experiment, and this strategy successfully produces chronic eczema symptoms. The patients received their oral medications for four weeks. The researchers chose a sample number which matched previous studies about immunology and dermatology that used DNCB-induced eczema models and provided enough data to produce meaningful statistical results.

Dose Selection and Treatment Protocol

The optimal immunomodulatory dose for treating chronic eczema through their analysis of existing pharmacological research and OECD-423 acute toxicity standards. Datura metel leaf ethanolic extract was administered orally at a dose of 10 mg/kg body weight/day, while Tephrosia purpurea root ethanolic extract was administered at 200 mg/kg body weight/day for 28 consecutive days. The 1:20 combination of Datura and Tephrosia extracts showed immunomodulatory properties which controlled T-cell disease advancement by reducing Th2 cytokines while preserving immune function without causing any side effects.

Treatment Protocol

Group	Exposure	Treatment	Number of Rats
Group 1	Normal Control	normal	6
Group 2	DNCB Induced	No treatment	6
Group 3	DNCB Induced	Single Datura metel	6
Group 4	DNCB Induced	Single Tephrosia purpurea	6
Group 5	DNCB Induced	Combined Treatment	6

Blood sample collection

Adhesive tape was used to attach the forelegs and rear legs of each rat so that blood could be collected. At a 45° angle, a 40 X 0.8mm needle was inserted into the sternum to puncture the heart. A little over 2.5 milliliters of cardiac blood were taken out. The blood was transferred into a sterile container with the anticoagulant EDTA after the

needle was quickly withdrawn. The laboratory team gained access to analyze T-cells and cytokines present in the blood sample.

RESULTS

Assessment of T-cell Activity

The optical density (OD) measurements at 570 nm were used to evaluate Concanavalin A (ConA) stimulated lymphocyte proliferation in every experimental group. The normal control group showed their usual T-cell proliferation pattern through an OD value of 0.77 ± 0.06. The T-cell proliferation of the untreated group which received DNCB treatment became significantly higher because their OD values reached 1.43 ± 0.09. The T-cell proliferation of Group 3 decreased after they took Datura metel ethanolic extract by mouth because their OD value became 1.08 ± 0.07. The ethanolic extract of Tephrosia purpurea (Group 4) treatment led to decreased T-cell proliferation which produced an OD value of 1.14 ± 0.06. The Group 5 recipients who received the combined treatment experienced their T-cell proliferation decrease to an OD value of 0.92 ± 0.08. The table in Table 1 contains all the necessary values.

ANALYSIS OF T-CELL SUBSET DISTRIBUTION

$CD4^+$ and $CD8^+$ T-cell percentages in peripheral blood samples were determined using flow cytometric analysis. In the normal control group, the levels of $CD4^+$ T-cells were 43.1 ± 3.2%, while those of $CD8^+$ T-cells were 32.5 ± 2.4%. In the group that was untreated and induced by DNCB, the percentages of $CD4^+$ T-cells rose to 57.6 ± 4.2%, whereas those of $CD8^+$ T-cells were noted to be 44.2 ± 3.7%. $CD4^+$ T-cell levels were at 47.3 ± 3.0% and $CD8^+$ T-cell levels were at 38.1 ± 2.5% following treatment with an ethanolic extract of Datura metel. The $CD4^+$ T-cell levels in animals treated with ethanolic extract of Tephrosia purpurea were 50.4 ± 3.5%, while the $CD8^+$ T-cell levels were 41.7 ± 3.1%. The $CD4^+$ T-cell levels in the combined treatment group reached 45.8 ± 2.8% while $CD8^+$ T-cell levels reached 35.2 ± 2.9%. The table contains complete data which appears in Table 1.

Table 1 T-cell Activity Assessment

Group	T-cell Proliferation (OD 570 nm)	CD4+ T-cell (Percentage)	CD8+ T-cell (Percentage)
Group 1 (Normal Control)	0.77 ± 0.06	43.1 ± 3.2%	32.5 ± 2.4%
Group 2 (DNCB Induced)	1.43 ± 0.09	57.6 ± 4.2%	44.2 ± 3.7%
Group 3 (Datura metel)	1.08 ± 0.07	47.3 ± 3.0%	38.1 ± 2.5%
Group 4 (Tephrosia purpurea)	1.14 ± 0.06	50.4 ± 3.5%	41.7 ± 3.1%
Group 5 (Combined Treatment)	0.92 ± 0.08	45.8 ± 2.8%	35.2 ± 2.9%

Cytokine Analysis:

Evaluate the immune system changes between experimental groups through serum cytokine level assessments. The group that was untreated and induced by DNCB showed increased levels of IL-4 and IL-13, which were measured at 18.5 ± 1.2 pg/mL and 20.3 ± 1.0 pg/mL, respectively. The research team measured IL-17 levels in the same group which amounted to 4.5 ± 0.3 pg/mL. The IL-4 levels in normal control subjects were measured at 5.1 ± 0.5 pg/mL while IL-13 levels were 6.2 ± 0.4 pg/mL and IL-17 levels were 13.5 ± 0.7 pg/mL. The IL-4 and IL-13 and IL-17 levels after Datura metel ethanoic extract treatment measured at 10.3 ± 0.8 pg/mL and 12.2 ± 0.7 pg/mL and 8.9 ± 0.6 pg/mL respectively. The IL-4 and IL-13 and IL-17 levels in Tephrosia purpurea ethanolic extract-treated animals were measured at 10.7 ± 0.9 pg/mL, 13.1 ± 0.8 pg/mL and 9.1 ± 0.5 pg/mL respectively. The IL-4 levels in the combined treatment group measured at 6.8 ± 0.6 pg/mL while IL-13 levels reached 7.5 ± 0.4 pg/mL and IL-17 levels reached 14.5 ± 0.8 pg/mL. The table below presents cytokine measurement results for all study groups

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Table 2 Cytokine Profiling Data

Group	IL-4 (pg/mL)	IL-13 (pg/mL)	IL-17 (pg/mL)
Group 1	5.1 ± 0.5	6.2 ± 0.4	13.5 ± 0.7
Group 2	18.5 ± 1.2	20.3 ± 1.0	4.5 ± 0.3
Group 3	10.3 ± 0.8	12.2 ± 0.7	8.9 ± 0.6
Group 4	10.7 ± 0.9	13.1 ± 0.8	9.1 ± 0.5
Group 5	6.8 ± 0.6	7.5 ± 0.4	14.5 ± 0.8

Histopathological Evaluation

The histological analysis of dorsal skin tissue from normal control animals showed that the epidermal layer remained intact while the epidermal thickness stayed consistent and the tissue showed only a few inflammatory cells. The untreated group that received DNCB induction developed severe skin changes which included thickened epidermis, swollen dermis, skin cell invasion by inflammatory cells and complete destruction of skin structure. The individual treatment of Datura metel and Tephrosia purpurea produced beneficial effects through changes which affected both epidermal thickness and inflammatory cell numbers. The group that received the combined treatment exhibited an epidermal organization close to normal, diminished inflammation of the dermis, and enhanced skin integrity. The skin barrier function improves because the epidermal structure returns to its natural state while the number of inflammatory cells in the dermis decreases. The severity of chronic eczema and its potential for recurrence depends on the function of the skin barrier system.

Oxidative Stress Parameters

The biochemical tests showed that MDA levels increased while superoxide dismutase and catalase activities and reduced glutathione levels decreased in the DNCB-induced untreated group. The treatment of individual plant extracts resulted in decreased MDA levels but the antioxidant enzyme activities only partially returned to their original state. The MDA levels in the combined treatment group remained lower than the individual treatment groups while their antioxidant enzyme activities reached higher levels.

Safety and Toxicological Considerations

The research used an optimized low-dose protocol which followed OECD-423 toxicity guidelines and previous pharmacological studies despite known toxic effects of Datura species. The treatment duration brought no fatal outcomes and no behavioral issues or physical side effects emerged during the study. The study results show no toxicity which proves that proper dosing methods are essential for safe long-term treatment with this specific extract combination.

DISCUSSION

The current study offers persuasive proof that, in a DNCB-induced chronic eczema model, the combined use of Datura metel and Tephrosia purpurea exerts strong immunomodulatory effects. The immune system continues to activate while T-cells become dysfunctional and Th2 cytokines rise and the skin barrier fails and oxidative stress increases which leads to chronic eczema development. The DNCB-induced group developed chronic immune-mediated skin inflammation which caused T-cell proliferation to rise while their $CD4^+/CD8^+$ ratios became different from normal values. The individual plant extracts used for treatment showed their ability to decrease T-cell proliferation because Datura metel and Tephrosia purpurea contain bioactive compounds which affect immune system operation. The combined treatment produced better results than either treatment alone because their phytoconstituents worked together in a synergistic manner. The combined treatment group reached immune equilibrium between $CD4^+$ and $CD8^+$ T-cells which demonstrated specific immune control instead of widespread immune system weakening that doctors need to treat chronic inflammatory diseases properly. The therapeutic efficacy became more apparent because the combined treatment approach successfully decreased IL-4 and IL-13 cytokine levels which showed that the treatment blocked Th2-mediated allergic inflammation. The restoration of IL-17 levels helped maintain Th2/Th17 immune equilibrium which stopped immune system fatigue and stopped the disease from getting worse. The current phytotherapeutic method provides better safety and multiple immunomodulatory effects than conventional anti-eczema treatments which include corticosteroids and calcineurin inhibitors that only treat symptoms but cause side effects when used for extended periods. The research demonstrated that the plant extract mixture brought back immune system operation without producing any adverse reactions which makes it an appropriate treatment for chronic eczema during prolonged therapy.

The body experiences oxidative stress which maintains inflammation while damaging its ability to heal tissues in patients with eczema. The combined treatment method reduced lipid peroxidation while it enhanced the body's natural antioxidant defenses which protect both immune system function and skin healing mechanisms. The histopathological examination results confirmed the biochemical and immunological test results because they showed that skin cells kept their typical appearance while showing reduced inflammation. Research studies have proven that plant-derived flavonoids and polyphenols show similar therapeutic effects which previous studies have documented for treating chronic inflammatory and autoimmune diseases. The main benefit of using multi-target phytotherapy instead of single-target conventional therapies exists because it controls multiple immune signaling pathways and oxidative stress and tissue repair mechanisms at the same time. The combined treatment produces better results because Datura metel alkaloids work together with Tephrosia purpurea flavonoids and phenolics and glycosides to generate enhanced therapeutic effects.*

Proposed Immunomodulatory Mechanism of Action

The immunomodulatory effects of Datura metel and Tephrosia purpurea treatment occur because these herbs control various immune system pathways which contribute to the development of chronic eczema. The alkaloids present in Datura metel plants interfere with cholinergic anti-inflammatory systems which results in lower T-cell activation and decreased production of IL-4 and IL-13 Th2

cytokines. The flavonoids and rotenoids present in Tephrosia purpurea serve as antioxidants which protect the immune system through their ability to stop NF-κB inflammatory pathways and help develop regulatory T cells (Tregs). The measured increase in IL-17 levels shows that the immune system restored its Th17-mediated balance which defends against immune system fatigue and continuous tissue destruction. The combined extract functions through multiple synergistic mechanisms which minimize harmful immune responses while maintaining beneficial immune system functions to produce better therapeutic outcomes than using each compound separately. The research shows NF-κB inhibition works together with Th2 cytokine suppression and Treg stabilization to control the immune system but it did not assess gene and protein expression levels. The study produced identical results through immunological and biochemical and histopathological tests which confirm the proposed biological mechanisms. Future research using molecular and transcriptomic methods will help scientists identify the exact signaling pathways which occur.

Statistical analysis

All experimental data are presented as mean ± standard deviation (SD). The statistical analysis used GraphPad Prism software version 9.0 for its operations. To compare the various experimental groups, a one-way analysis of variance (ANOVA) was conducted, followed by Tukey's post-hoc test for multiple comparisons. The study established statistical significance for group differences when p values reached less than 0.05. The research data showed that T-cell function together with IL-4 and IL-13 and IL-17 cytokine levels reached their highest points in the group which received both treatments.

Limitations of the Study

The research contains specific restrictions which affect its findings. The research failed to verify immune signaling pathways through molecular evidence which involved studying transcription factor expression and gene regulation patterns. The research study did not include quantitative tests to measure phytochemical compounds. The therapeutic potential of this combination therapy needs additional validation through clinical studies and molecular assays and pharmacokinetic evaluations.

CONCLUSION

The research conducts a complete experimental evaluation of Datura metel and Tephrosia purpurea ethanolic extracts as immunomodulatory agents for treating chronic eczema through studies on DNCB-induced rat models which duplicate human atopic dermatitis immunological patterns. The study examines plant-derived bioactive compounds which affect T-cell immune system dysregulation and skin inflammation through complete phytochemical analysis and multiple tests that include immunological and biochemical and histopathological and toxicity evaluations. The research focused on identifying core immune system mechanisms which cause the disease by developing strategies to restore T-cell and cytokine equilibrium in eczema patients.

The research results showed that patients achieved superior therapeutic results when Datura metel and Tephrosia purpurea were used together instead of separately. The treatment approach successfully controlled uncontrolled T-cell growth while it established proper $CD4^+$ and $CD8^+$ T-cell numbers and it decreased Th2 cytokines IL-4 and IL-13 while it brought back IL-17 levels to normal. The protective effect resulted from immunological control which simultaneously reduced lipid peroxidation to decrease oxidative stress and strengthened the body's natural antioxidant defenses. The process needed to protect skin tissue from developing long-term inflammatory injuries. The histopathological results showed that the epidermal thickness decreased while inflammatory cells decreased in number and the skin structure returned to normal which supported the biochemical and immunological improvements. The research on dose validation and toxicity proved that the selected doses were safe for patients because they did not cause any negative side effects. The research creates a complete experimental system which proves Datura metel and Tephrosia purpurea function as natural immunomodulators which affect multiple biological pathways to treat chronic eczema. The research findings confirm the statements from traditional medicine while establishing scientific backing for plant-based immunomodulatory treatments which provide safer options than standard medical treatments. The development of safe and effective treatment methods for human patients with chronic inflammatory skin diseases needs additional clinical trials and pharmacokinetic studies and formulation-based research.

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